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EFFECTS OF THE EXTIRPATION OF THE ANTERIOR LOBE OF THE HYPOPHYSIS OF RANA PIPIENS.

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These experiments were performed in the spring of 1916, although attempts had been made the previous year which failed because of faulty technique.

Adler ('14) removed the hypophysis ingrowth by using the electric cautery at a stage of 20 mm. length. This caused immense mortality and he was able to rear only 3 specimens. In these the hind limbs did not grow beyond the condition of mere buds and persisted in this condition long after metamorphosis of the controls and many months after the operation. He found that the growth of the thyroid gland was markedly retarded by the removal of the hypophysis and the amount of colloid was much reduced.

P. E. Smith performed quite similar experiments simultaneously with mine. Both of us gave simultaneous accounts of our respective researches before the San Diego meeting of the Western Society of Naturalists August 9 to 12. He published his results in *Science*, August 25, 1916, and in the *Anatomical Record*, October, 1916. I gave a preliminary account of my work in *Science* November 24, 1916. Since I have been delayed in making this more complete report, I can allow myself the privilege of drawing comparisons between Dr. Smith's work and my own.

My account of the experiments upon the hypophysis was delayed owing to the fact that I was absent from Lawrence during the summer and was unable to complete the study of my material until my return in September. I had also hoped to combine the

account of these experiments with a full account of my work upon thyroid removal. Since six of the tadpoles in the latter experiment are still alive and the work upon the preserved material far from complete it seemed after all advisable to publish separate papers upon the two lines of work.

In the present series of experiments the removal of the hypophysis ingrowth was accomplished by making a frontal cut just

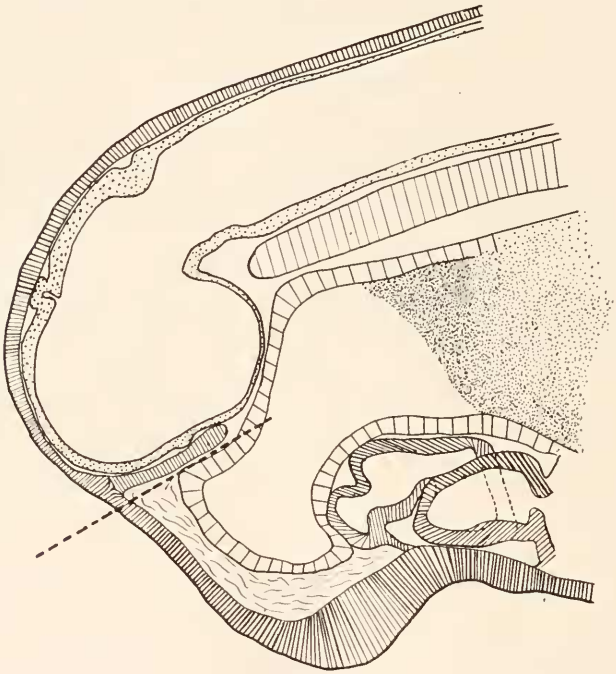

FIG. 1.

beneath it and extending more than its entire length as shown in Fig. 1. It was then quite a simple matter to open up the wound and remove the hypophysis ingrowth by means of a spear-point needle. This was performed under a binocular microscope. The stage chosen for operating is that of 3.5 to 4 mm., the operation being performed more readily at this time than later because at this stage the ingrowth is compact and thick, but later becomes thinned out and more closely applied to the diencephalon, thus increasing the difficulty experienced in

seeing and removing it. In each case it was broken away from the ectoderm to which it was still attached at the stage at which the operation was performed. In most instances the dorsal portion of the pharynx was partly removed; but, as will be seen later, this did not appear to affect the formation of the thymus glands.

The tadpoles recovered very quickly from the operation, healing of the wound taking place in the course of 20 or 30 minutes, after which the tadpoles became as active as ever and appeared to be quite normal. In about 1/3 of the cases the anlage of the upper part of the mouth was so injured by the operation that it developed imperfectly, hence these individuals were doomed to starve. Observations upon this point were made in the following lots as indicated:

Experiment.	Length of Tadpole at Time of Operation.	No. with Perfect Mouth.	No. with Defective Mouth.	Total No. Examined.
No. 23....	3.5–4 mm.	14	6	20
No. 24....	3 –3.5 mm.	22	11	33
No. 27....	3 –3.5 mm.	19	16	35

The operated tadpoles grew quite normally resembling the controls in every regard until 7 or 8 days after the operation when they were 8 mm. in length. They then underwent a striking color change from the earlier solid black to a bright creamy silver color, becoming really quite handsome creatures. In the controls many pigment cells are found in the lower layer of the epidermis, where they give off a rich network of processes. In sharp contrast to this is the fact that in the tadpoles deprived of the hypophysis only very seldom can a much contracted pigment cell be demonstrated in the epidermis. It seems quite certain that the pigment cells have migrated to deeper positions. In both the controls and the operated tadpoles they are found in great numbers on the surface of the skull, the inner surface of the gill cavity, the outer surface of the heart, within the brain, and spinal cord, upon the gills, in the pericardium, very extensively in the peritoneum, in the pronephros, liver, thyroid, lungs, intestine and walls of the blood vessels, but there is a constant difference in that they are expanded in the normal

tadpoles and much contracted in the operated ones. It thus seems fair to conclude that in the absence of the hypophysis they migrate inward from the epidermis and that they contract throughout all parts of the body. In the more superficial cells of the epidermis scattered pigment granules are found here and there during later stages up to and beyond the 15 mm. stage. These persist in both the operated and control tadpoles. These observations and a careful study of the pigment cells convince me that there is no disappearance and bleaching of pigment granules as asserted by Smith. I differ from him further in my observations of the contraction of the pigment cells in the interior of the body in the operated tadpoles.

FIG. 2.

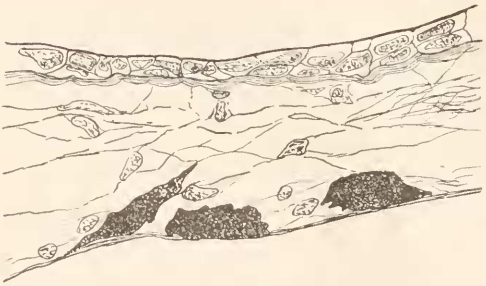

FIG. 3.

FIG. 4.

Figs. 2, 3, and 4 are all drawn to scale. Fig. 2 represents a lateral portion of the body wall of a normal control tadpole. The pigment cells can be clearly distinguished because of their darker color. Fig. 3 represents a similar portion of the body wall of a tadpole from which the hypophysis has been removed. This shows the absence of pigment cells in the epidermis and the contraction of those in the peritoneum. Fig. 4 shows a surface view of a pigment cell in the peritoneum of an operated tadpole.

We shall now take up a detailed account of the different experiments that bear upon the subject of this paper.

No. 13.—April 9 and 10, 1916. Length at time of operation 5.5 mm. These tadpoles were in too advanced a stage of development to give satisfactory results. It was impossible to clearly see the hypophysis ingrowth because it had become so much broadened and flattened. The ventral surface of the forebrain was scraped and frequently injured in the process. Under this heading are included three series of experiments.

(*a*) April 9. 14 operated, rather crudely. 10 remained alive at the end of 9 days. These were discarded because of later improvements in technique.

(*b*) April 9. 12 operated. The work was much more carefully performed than in (*a*). The under side of the fore brain was carefully scraped and all specimens that showed injury to the brain were discarded. 6 remained alive at the end of 11 days when they were preserved for sectioning.

(*c*) April 10. 20 operated. In this case the floor of the diencephalon behind the optic stalks was removed in order to insure the complete removal of the hypophysis which in itself is quite hard to recognize at this late stage. Only 3 remained alive at the end of 6 days and they were distorted.

These experiments were made at entirely too late a stage of development to be successful. They are merely given to show the stages through which the work progressed.

No. 14.—(*a*) April 10. 12 operated. 3 alive at the end of 6 days. Length at time of operation 4 mm. This was the first really successful experiment. This unfavorable result was purely due to neglect owing to the press of work in later experiments. The remaining specimens were killed.

(*b*) April 11. 65 operated. Tadpoles kept in a finger bowl for 2 days when it was found that 20 showed abnormalities due to lack of oxygen. These were isolated, some dying within a few days while others partly recovered. These were later discarded. Of the remaining 36 healthy tadpoles 31 were alive at the end of 26 days. During this time they were kept in soft cistern water. They were fed algae which helped to oxygenate the water in the aquaria. The close quarters of the aquarium

bowl in which they were kept no doubt retarded their growth. They showed the following characters as to color and size:

	No. Specimens.
Dark but with a more silvery cast than usual — 11, 12, 12, 13 and 14 mm. — one each	5
Silvery runts under 11.5 mm.	16
Silvery specimens of larger size—11.5–13.5 mm.	7
14, 16, and 18 mm. — one each	3
All but two of the small ones had a poorly formed mouth. Killed May 7	31

(*c*) April 12. 51 operated. Of these, 8 were used for experiment 17. Of the remaining 43, two died on April 29; one on the 30th and then the mortality became very marked so much so that 31 died in the succeeding 5 days. On May 5 the remaining 9 were preserved. They had lived 23 days. These were kept in city water in roomy kitchen sink aquaria from April 18 on. The high mortality in comparison with that of lot 14 (*b*) is really very striking.

No. 17.—April 12 hypophysis removed, April 13 thyroid also removed. Kept throughout in a glass bowl aquarium in soft cistern water. At the end of 31 days two were dead, the remainder were killed. Of these killed May 13, 5 were light and 1 dark.

No. 19.—April 14. Length at time of operation 5 and 6 mm. 21 operated. The operation was performed upon specimens too far advanced. They showed a high rate of mortality and were soon discarded.

No. 22.—April 20. Stage at operation varied 3.5–4.5. 40 operated. Two died soon after. Divided into two lots of 19 each. Lot (*a*) was kept as before, in lot (*b*) the thyroid gland was also removed.

In Lot (*a*) 3 dead by May 1.

In Lot (*b*) 3 dead by May 3.

All dead in few succeeding days. Data not complete. On April 28 both (*a*) and (*b*) equally showed the characteristic color change due to removal of the hypophysis proving that this is not due to any lack of balance between these two glands.

Controls kept in same sink showed no mortality.

No. 23.—April 22. Stage of operation 3.5–4 mm. 42 operated. Great care was exercised to avoid injury to the brain.

April 29. 7 died.
April 30. 5 died.
May 1. 5 died.
May 2. 5 died.

On May 2, 19 were nearly dead—only the faintest heart beat could be seen and only 1 was still active. The 20 were killed on that date.

Controls kept in neighboring sink showed no mortality.

No. 24.—April 23. Stage of operation 3–3.5 mm. 51 operated. Great care was exercised to avoid injury to the brain.

April 29. 1 died.
May 1. 3 died.
May 2 and 3. 36 died.

Only 11 remained alive at this time. Controls kept in neighboring sink showed no mortality.

No. 27.—April 30. Length at time of operation 3.5 to 4 mm. 103 operated. 3 died within 24 hours. Care was exercised to avoid injury to the brain. Divided into two lots.

(*a*) 30 kept in aquarium bowl in soft water for 10 days; there was no mortality during this time. Transferred to sink with city water on May 10. May 21 all dead but one that was dark in color.

(*b*) 70 kept in sink with city water from outset.

May 4	3	dead
" 6	1	"
" 16	10	"
" 17	10	"
" 18	13	"
" 19	7	"
" 21	8	"
" 24	9	"
June 1–10	3	"
	64	dead
Missing	2	
Killed June 1	1	
Killed June 10, of these, 1 black, 1 gray, 1 white (small)	3	

Controls showed no mortality during this time.

No. 28.—May 8. Length at time of operation 3.5–4 mm. 30 operated. On June 10 4 remained alive—two killed and two preserved. One of these lived until July 2 while the other lived

until Aug. 30th. The former was killed when 24 mm. long. The latter reached a length of 30 mm. It was extremely active and of a bright silver color. It was unfortunately lost.

TABLE SHOWING MORTALITY IN VARIOUS EXPERIMENTS.

In Soft Water.

No. of Exp.	Time of Starting.	Termination of Experiment.	No. Days.	No. Tadpoles in Exp.	No. Dead.
14 *b*.....	April 11	May 7	26	36	5
17.....	April 12 and 13	May 13	30	8	2
		In City Water (Hard).			
14 *c*.....	April 12	May 5	23	43	34
23.....	April 22	May 3	11	42	41
24.....	April 23	May 3	10	51	40
27.....	April 30	June 1	31	103	97
28.....	May 8	June 10	32	30	26

We can see in these experiments the serious effect of the Lawrence city water upon tadpoles deprived of the anterior lobe of the hypophysis. An analysis of the water made June 1 shows the following mineral content:

In terms of mg. per liter.

Total solids	584.0	
SiO_2	26.2	
Fe_2O_3—$A(2O_2$	2.6	fluctuates greatly from this figure to 13 mg.
Ca	114.8	
Mg	16.0	
Na plus K	62.8	
Cl	78.0	at no time during experiment in free condition.
SO_4	58.8	
HCO_3	388.0	

The Fe is the only ingredient that shows frequent fluctuation. It is interesting to note in this connection the results of a test control experiment.

No. 27 X.—On May 1 tadpoles from the same batch used in 27 were employed. These had been held in their development at the operative stage by being kept upon ice. Soon after the operation they were placed in city water.

(*a*) 16 tadpoles were cut along a plane lying just above the hypophysis ingrowth. In the majority of cases the brain was touched. 4 died on May 5.

(*b*) 15 tadpoles were cut in the usual manner in preparation for removal of the hypophysis ingrowth but as in the preceding (*a*) the hypophysis ingrowth itself was left intact.

After (*a*) and (*b*) had been kept separate for 10 days with the loss of 4 in (*a*) they were then combined.

Following this, deaths occurred as follows:

May 18	1 dead
" 21	1 "
" 24	2 "
" 25	5 "
" 25–June 9	6 "

Thus on June 9 14 were still living and healthy. They were then killed. It is interesting to compare the results obtained here with those found in the above table. It is seen that by deducting the 4 that died in the first 5 days, as a result of serious injury there were 28 that had a good start without any mortality until May 18; 14 or $\frac{1}{2}$ of these survived 40 days later. Unfortunately a study of the mouths of these tadpoles was not made. If it had been done it might have been possible to explain some of this mortality as a result of starvation due to imperfect mouth development resulting from injury. A series of measurements made on May 16 showed 12 to be below 11.5 mm. in length while the maximum length was 13.6 mm. The very smallest, 8.7 mm., was of a silvery color due supposedly to the accidental removal or injury of the hypophysis ingrowth.

This leads to the important point that this was the only case of a color change in the 28 specimens, thus showing that the absence of the hypophysis and not the mere wound itself is the cause of the color change. Along this line may be mentioned that in nearly every experiment where removal of the hypophysis was practiced one or two operated tadpoles failed to show the color change characteristic of the others. Several of these were sectioned and in each case it was found that there had been failure to remove the hypophysis. There is thus no doubt whatever that the absence of this gland is the cause of it. The nature of

this color change will be discussed later. The operation and the absence of the hypophysis have their influence upon the size of the tadpoles.

Lots 27(*a*) and (*b*) also 27 X (control operated) (*a*) and (*b*) were measured on May 16 giving the following results:

Lot.	Maximum.	Minimum.	Average.	No. Specimens.
(27 *a* (deprived of hypophysis)....	12.0 (dark) 10.6	8.3	9.43	15
27 *b* (deprived of hypophysis).....	15.3	8.9	10.74	45
27 X *a* and *b*—operated control...	13.6	8.7 silver one 9.6 dark	11.53	29
Control (none operated).....	20.4	11.6	16.95	30

It is to be remembered that 27 X *a* and *b* was performed 1 day later than 27 *a* and *b*.

It is thus seen that although the operated controls did not grow as rapidly as the unoperated controls they grew decidedly more rapidly than did the operated tadpoles. It is also seen that a few of the largest operated tadpoles showed greater size than the smaller controls. It is difficult to explain this. It may be partly due to a difference in vitality.

The serious mortality and delay of growth are in sharp contrast with the results of the equally severe operation of removal of the thyroid anlage. This was performed by making a transverse cut between the heart and the thyroid, and then picking out the latter by means of a needle. The cut was quite as large as that made for the removal of the hypophysis and produced a considerable loss of blood, yet there was no greater mortality in the operated tadpoles than among the controls. In time they appeared precisely like the normal controls and remained so until the legs began to grow in the controls while remaining at an early stage of development in the thyroidless tadpoles.

Returning to the experiments upon removal of the hypophysis, upon the question of mortality my results are quite at variance with those of Dr. Smith who found that there was even less mortality among his operated tadpoles than among his controls. I feel that this difference between his results and mine may well be explained by the fact that our material was reared in a different water supply. It is quite significant that lots 15 *b* and 17 raised

in aquarium jars with soft cistern water lived with very little mortality until I killed them. They had ceased to grow because of overcrowding in narrow quarters. The serious effects of our water supply upon normal tadpoles were noted in a series of experiments upon removal of the thyroid, to be published later. In these both the thyroidless tadpoles and their controls began to show a remarkable twist in their tails when they reached a length of about 25 mm. on June 8, 57 days after the operation. At this time they began to show a high mortality. This was even more marked in the controls than in the operated ones. Only a very few that had been deprived of the hypophysis remained alive at this time and they were not far enough advanced to show these modifications.

This whole matter will be the subject of extensive work next spring in an effort to determine whether the high mortality among the tadpoles deprived of the hypophysis is due to a resulting susceptibility to some specific substance in the water. We should be able by these means to trace out some features in the influence of the hypophysis upon metabolism.

There was marked retardation in the development of the limb buds. This was quite evident in the 24 mm. specimen killed July 2. The operated tadpole of this stage had limb buds 234 micra while in the control they were 532 micra in length. According to the reports of a friend who kept the 30 mm. tadpole under observation up to the time of its death, August 30, the limb buds remained strictly rudimentary. At this time the controls had long before undergone metamorphosis. In these regards my work is in complete accord with that of Adler and Smith, namely, the removal of the hypophysis prevents the hind legs from developing beyond a rudimentary condition, and causes the tadpoles to remain in a larval state.

The internal structure of the few operated tadpoles that reached sufficient size was studied in comparison with the controls. The stages thus investigated were of 15.5 mm., 16.5 mm., 21.5 mm., and 24 mm. length. One that reached 30 mm. length unfortunately died without being preserved. Especial attention was given to a study of the thyroid and thymus glands and to the gonads. A more cursory examination of other organs was made, but failed to show any noteworthy features.

In measuring the different organs there were in each case three measurements made in portions that show approximately maximum thickness. Such measurements were made in two dimensions in each case. The averages computed from them are as follows:

16.5 Mm. Stage.

	Thymus.			Thyroid.			Gonad.		
	Leng., μ.	Bread., μ.	Thick., μ.	Leng., μ.	Bread., μ.	Thick., μ.	Leng., μ.	Bread., μ.	Thick., μ.
(Control, indif. sex)									
Left	420	248	362	150	114	21	710	78	78
Right	560	234	476	150	78	21	610	64	85
(Operated, indif. sex)									
Left	430	341	532	150	106	44	790	64	135
Right	420	341	504	120	114	35	630	94	99

21.5 Mm. Stage.

(Control, female)									
Left	660	327	461	220	142	21	1,150	85	170
Right	670	335	639	210	111	22	990	135	142
(Operated, male probably)									
Left	460	369	639	190	170	43	930	59	67
Right	470	412	882	200	170	43	790	67	75

24 Mm. Stage.

(Control, male probably)									
Left	840	426	639	290	177	57	1,170	114	99
Right	880	426	639	300	149	57	1,020	114	114
(Operated, male)									
Left	790	525	852	250	270	28	660	114	170
Right	860	504	696	210	362	28	840	114	92

TABLE SHOWING THE NUMBER OF FOLLICLES IN THE THYROID GLAND AND THE AVERAGE DIAMETER OF THE COLLOID SECRETION MASSES.

16.5 Mm. Stage.

		Number of Colloid Masses.	Diameter of Colloid Masses.
Control	Left	20	
	Right	12	
Operated	Left	15	
	Right	16	

21.5 Mm. Stage.

Control	Left	16	22μ
	Right	13	
Operated	Left	7	11μ
	Right	8	

24 Mm. Stage

Control	Left	21	29μ
	Right	25	
Operated	Left	20	18μ
	Right	17	

It is clearly evident from the above figures that tadpoles deprived of the hypophysis produce far less colloid substance in their thyroid glands than do the normal controls. This is a markedly constant point of difference. This would seem to be an index of its secretory activity in the two cases; although the recent work of Bensley would cast doubt upon the value of the colloid accumulation as an index of the secretory activity of the thyroid.

In the 16.5 mm. and 21.5 mm. stages, the thyroid is actually larger in the operated individuals than in the corresponding controls while in the 24 mm. stage the thickness—dorso-ventral dimension—is on the contrary almost twice as great in the control as in the operated specimen. The material is too scanty to enable one to draw positive conclusions upon this score from my work alone. It may be well to point out however that Adler, Smith and myself are in agreement regarding the effect of the removal of the hypophysis upon the development of the thyroid gland and regarding the relatively small amount of colloid produced in them.

I should hesitate to attribute the failure of the limbs to develop in operated tadpoles to the lessened activity of the thyroid resulting from the absence of the hypophysis. This question must be tested by more crucial experiments than any as yet applied.

In general the thymus gland is larger in the operated tadpoles than in the controls. The differences are not constant nor are they striking in the face of the general variability of this organ. It only seems safe to state that the thymus is not adversely affected in size or structure by the removal of the hypophysis.

The gonads show a large amount of variation in size and form. The absence of the hypophysis does not produce any apparent modification in them in the stages studied. The germ cells, sex cords and the various structures of the gonads appear to be quite unmodified by the operation. The operated tadpoles of 21.5 mm. and of 24 mm. stages after sexual differentiation are both males. It is thus impossible to tell whether there is any difference in the effects upon the two sexes. This point of the effect upon the gonads must be carried out in future experiments reaching to much later stages and involving far larger numbers of tadpoles.

Summary.

1. The removal of the anlage of the anterior lobe of the hypophysis early causes the pigment cells to contract, and those of the epidermis to withdraw from it into the interior. This takes place while the gland shows little or no apparent histological differentiation.

2. Evidence has been given to show that the absence of the hypophysis in tadpoles makes them highly susceptible to unfavorable chemical conditions of the water. This will be tested out specifically in later experiments.

3. The absence of the hypophysis causes—either directly or indirectly—a failure to undergo metamorphosis especially evident in the absence of limb growth beyond a very rudimentary condition.

4. The absence of the hypophysis causes a marked diminution of colloid formation in the thyroid gland and in the stage of 24 mm. involves a retardation in its growth.

5. During the stages studied the absence of the hypophysis produced no noticeable effects upon the thymus gland nor upon the gonads.

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